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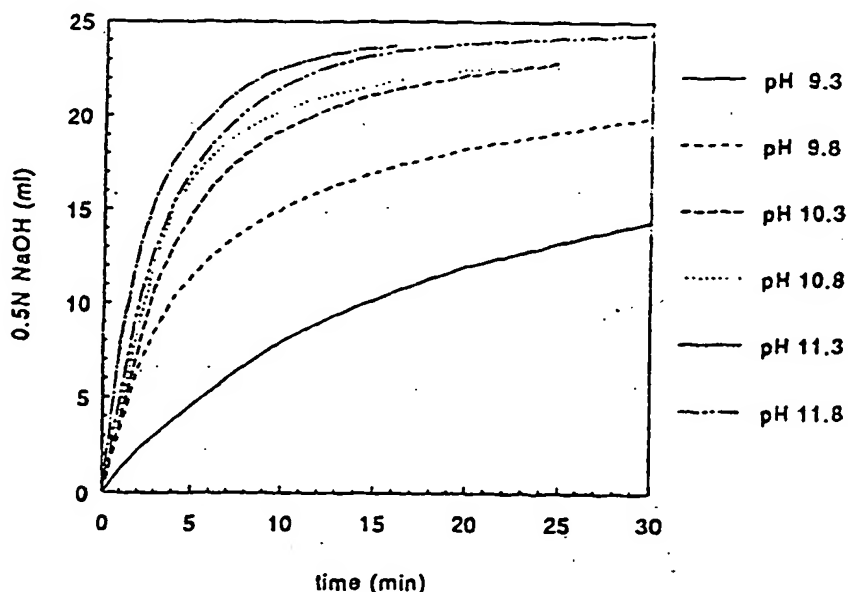
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(54) Title: METHOD FOR OXIDISING CARBOHYDRATES



(57) Abstract

Method for oxidising carbohydrates having a primary hydroxyl group, such as starch, inulin and fractions and derivatives thereof, by treatment with hypobalite in the presence of a catalytic amount of ditertiary-alkyl nitroxyl, in particular 2,2,6,6-tetramethylpiperidin-1-oxyl, in a water-containing medium at pH 9-13. The catalytic amount of nitroxyl is, in particular, 0.1-2.5 % by weight based on the carbohydrate. The oxidation leads to products having a high content (> 90 %) of carboxyl groups, without significant chain breakdown.

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Method for oxidising carbohydrates

The invention relates to a method for oxidising carbohydrates which contain a primary hydroxyl group, in the presence of a nitrogen oxide compound.

- 5 The oxidation of carbohydrates such as starch and cellulose is important because it allows a modification of the properties of the carbohydrates in a desired direction. Thus, oxidised carbohydrates can be used, inter alia, as thickeners, gelling agents, binders, swelling agents, stabilisers and complexing agents (phosphate substitutes).
- 10 Most processes for the oxidation of polymeric carbohydrates involve a greater or lesser degree of unwanted depolymerisation (hydrolysis). Nor, furthermore, is such an oxidation always specific: thus starch can be oxidised both on the primary hydroxyl group, at the 6-position, which leads to a carboxyl starch having an intact carbon skeleton, and
- 15 on the secondary hydroxyl groups on the 2,3-positions, which leads to the carbon-carbon chain in the glucose units being broken ("dicarboxyl starch").

- Oxidised carbohydrates having an intact carbon skeleton, i.e. carbohydrates oxidised at the primary hydroxyl function, in general
- 20 designated as polyuronic acids, are often advantageous for certain applications, for example as a complexing agent or a stabiliser.

- Davis and Flitsch, *Tetrahedron Lett.* 34, 1181-1184 (1993), describe the oxidation of monosaccharides wherein the non-primary hydroxyl groups are partly protected, using sodium hypochlorite.
- 25 potassium bromide and 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) in a two-phase solvent system (dichloromethane and water). The highest reported crude yield is 83% for 1-O-methyl-2-O-benzyl-3-O-acetyl-glucuronic acid.

- A method for oxidising polysaccharides, in which an excess of
- 30 sodium nitrite is used, is known from the work of T.J. Painter et al. (*Carbohydrate Res.* 55, 95-103 (1977), *ibid.* 140, 61-68 (1985)). According to this known method, cellulose or amylose is oxidised with sodium nitrite in phosphoric acid. A product is obtained in the process which, in the case of the oxidation of cellulose, has a
- 35 content of 87.5% of glucuronic acid and, in the case of oxidation of

amylose, with a yield of 66 and 86%, respectively, has a content of glucuronic acid of 67-75% and 52-56%, respectively.

A drawback of this known method is the high consumption of oxidant and the long reaction time (24 hours or more). Moreover, the reaction mixture must satisfy certain requirements with regard to viscosity and foaming. Furthermore, a higher effective yield is desired.

WO 91/04988 discloses a method for producing polyglucuronic acids by oxidising glucans electrochemically or with nitrogen oxides in the presence of a complexing agent such as lauric acid. The yields reported are moderate: 66% with an uronic acid content of up to 75%.

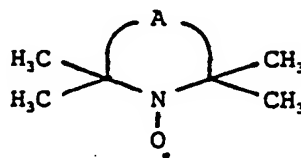
A method has now been found which does not have the drawbacks mentioned and which surprisingly has a higher specificity and selectivity (for oxidation of primary alcohol functions vs. oxidation of secondary alcohol functions which are usually more abundant in carbohydrates) than the known oxidations.

The method according to the invention for oxidising carbohydrates is performed by means of a hypohalite in the presence of a catalytic amount of a di-tertiary-alkyl nitroxyl and is characterised in that the carbohydrate is oxidised in an aqueous reaction medium at a pH of between 9 and 13.

The method according to the invention makes it possible to obtain, in a considerably shorter time (a few minutes), consuming no more than an approximately stoichiometric amount of oxidant, an oxidised carbohydrate which, in terms of degree of oxidation, selectivity of the oxidation and avoiding depolymerisation, is superior to the products of the known methods.

The di-tertiary-alkyl nitroxyl may be acyclic, but it is preferably a cyclic compound which satisfies the formula 1 below:

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In this formula, A represents a chain of preferably two or three atoms, in particular carbon atoms (methylene groups) or a

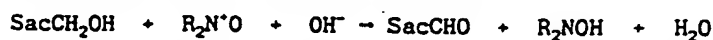
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combination of one or two carbon atoms with an oxygen or nitrogen atom. Chain A may, if required, be substituted by one or more groups such as alkyl, alkoxy, aryl, aryloxy, amino, amido or oxo groups, or by a divalent or multivalent group which is bound to one or more other groups having formula 1. The di-tert-alkyl nitroxyl may also be a part of a polymer structure such as $-\{(\text{CH}_3)_2\text{C}-\text{A}-(\text{CH}_3)_2\text{C}-\text{NO}^{\cdot-}\}_n-$.

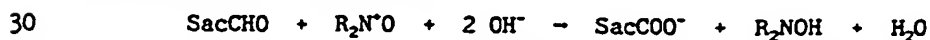
Highest preference is given, in the method according to the invention, to using 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO). The di-tert-alkyl nitroxyl may, if desired, be prepared in the reaction medium, for example by oxidation of the corresponding di-tert-alkylamine with hydrogen peroxide and tungstate.

The oxidation of alcohols with hypochlorite in the presence of nitroxyl compounds such as TEMPO is known per se. According to the known methods, however, simple alcohols only are oxidised, and a two-phase system is used in the process (see, for example, P.L. Anelli et al., *J. Org. Chem.* 54, 2970-2972 (1989)).

A catalytic amount of nitroxyl is understood to be an amount which - after conversion of the nitroxyl radical into the corresponding nitrosonium ion - is less than an amount required for oxidation of all the primary hydroxyl groups into carboxyl groups, in particular of less than 10% of the amount required for that oxidation, according to the following overall reaction equation:



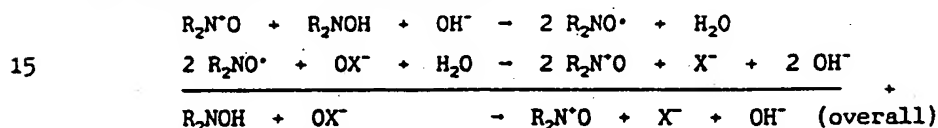
Here, Sac represents the radical of a monosaccharide unit, and R represents a tertiary alkyl group, with the possibility of the two groups R being linked together. The oxidation with nitroxyl to aldehyde is followed by oxidation of the aldehyde (possibly as its hydrate) to carboxylic acid, probably also by the nitrosonium species, according to the equation:



An amount of nitroxyl compound larger than 10% by weight does no harm, but is unattractive because of the higher costs. Preferably, the catalytic amount of nitroxyl is 0.005-5% by weight, more preferably 0.1-2.5% by weight, and especially 0.25-1.5% by weight, based on the carbohydrate. Expressed in mol.%, the catalytic amount of nitroxyl compound, based on the carbohydrate monomer, is preferably 0.1-2.5 mol.%.

The oxidation can be carried out in water, using a hypohalite as an oxidant, preferably in the form of a salt thereof, such as lithium hypochlorite, sodium hypochlorite, potassium hypochlorite or calcium hypochlorite. The amount of oxidant used is preferably 0.8-2 equivalents, preferably 0.9-1.5 equivalents and especially 1-1.2 equivalents. As a molar ratio, the amounts of oxidant to be used are 1.6-2, 1.8-2 and 2-2.4 mol per mol of monosaccharide unit, respectively.

It is assumed that the hypohalite reoxidises the hydroxylamine, formed during the oxidation of the alcohol function of the polysaccharide, to the corresponding nitroxyl radical or the corresponding nitrosonium ion $R_2N^+=O$, which subsequently is again able to oxidise an alcohol function, as shown in the reaction equations below, in which the hypohalite is represented by OX^- .



The hypohalite used can be hypochlorite, for example sodium hypochlorite. Advantageously, use may also be made of hypobromite which in that case is preferably obtained in the reaction medium from hypochlorite and bromide. In addition to an equivalent amount or a slight excess of hypochlorite a substoichiometric amount of bromide, or even a catalytic amount of bromide is sufficient in this case. Preferably, 0.2-1 equivalent (0.4-2 mol/mol of monosaccharide unit) of bromide is used. The use of hypobromite was found to result in improved yields of carboxysaccharides with less side-reactions.

The method according to the invention is carried out in a basic reaction medium with a pH of higher than 9 up to 13. In particular, a pH of 9.3-12 is employed, preferably a pH of 9.8-11.5. For polysaccharides, most preferably a pH of 10.3-11.5 is employed.

As can be seen from the net reaction equation:



one equivalent of base is consumed for each primary alcohol that is converted to carboxylate. The reaction can thus conveniently be followed by monitoring the base consumption at the pH selected.

The reaction temperature can vary from approximately -5°C to about 30°C. Preferably, the reaction is carried out at temperatures of below 10°C and more preferably at approximately 0-5°C.

The method according to the invention can be used for oxidising carbohydrates of very diverse types and origin (vegetable, animal, microbial, synthetic). Both monomeric carbohydrates (monosaccharides), and dimeric, oligomeric and polymeric carbohydrates, as well as sugar alcohols can be oxidised, if they have a primary alcohol function. Examples of polymeric carbohydrates are β -glucans, such as cellulose (1,4- β), curdlan and scleroglucan (1,3- β) and fractions, derivatives and hydrolysis products thereof, α -glucans, in particular starch (1,4- α) and pullulan (1,6/1,4/1,4- α) and fractions, derivatives and hydrolysis products thereof - such as amylose and amylopectin -, and cyclic equivalents thereof such as cyclodextrin, also other polysaccharides such as inulin (essentially a 2,1- β -fructan), and natural or artificial gums such as xanthan (1,4- β , with side chains), guar, carob flower, algin, gum arabic, dragacanth, agar, ghatti, chitin, carrageenin, and the like. In particular, the method is suitable for the oxidation of water-soluble oligosaccharides and polysaccharides such as starch or inulin, or fractions, hydrolysates or derivatives thereof. The preferred substrates for the present oxidation reaction are therefore α -glucans and fructans, especially α -glucans.

When oxidising monosaccharides or oligosaccharides having a cyclic hemiacetal as a terminal unit, it may be preferable to protect the (terminal) hemiacetal function e.g. by alkylation, so as to avoid any side reactions resulting e.g. in glycaric acid units. It was found however that with unprotected oligosaccharides having a chain length of about 15 and higher, in particular about 20 and higher, no detectable side reactions occur.

The method according to the invention can be employed for the production of completely carboxylated carbohydrates, in other words of polyuronic acids. The method can, however, also be used advantageously for preparing partially carboxylated carbohydrates, in which only some of the primary hydroxyl groups of the carbohydrate are oxidised. Preferably, carbohydrates having a carboxyl content of at least 75%, in particular of at least 90% are prepared. The invention also relates to polyuronic acids of a chain length of at least 15 monosaccharide units having an uronic acid content of at least 75%, or even at least 85%, in the case of polyfructans such as inulin, and of at least 90% in the case of poly- α -glucans such as starch derivatives. In the

latter case the poly- α -glucuronic acids preferably have a chain length of at least 20 anhydroglucose units and more preferably an uronic acid content of at least 93%.

After the reaction, the mixture can be worked up and the oxidised carbohydrate can be isolated by adding a solvent in which the inorganic substances dissolve and the product does not dissolve, for example an alcohol. Further purification can be carried out in a manner known per se. The yields of uronic acid in general are above 90%. The di-tertiary-alkyl nitroxyl used as the catalyst can be recovered from the reaction mixture, for example by extraction with an ether.

EXAMPLES

General

Potato starch (soluble in water, 21.5% amylose, 10% water) was obtained from Avebe, the Netherlands. Amylodextrin (degree of polymerisation 25) was obtained from waxy corn starch by means of pullulanase (see Dutch Patent 165500). 2,2,6,6-Tetramethylpiperidin-1-oxyl was of analytical grade (Sigma). Polygalacturonic acid (98%) was from Sigma; D₂O (99.9%) from Isotec Inc., and ethanol (96%) from Gist-Brocades.

NMR spectra were recorded by means of a VARIAN UNITY 400 spectrometer (¹H resonance frequency 400 MHz, ¹³C resonance frequency 101 MHz). ¹³C NMR spectra were recorded as "gated decoupled", allowing quantitative determination. All the samples were dissolved in D₂O. HPLC analysis was carried out by means of an Ultrapac TSK G5000PW column of 7.5 × 600 mm, coupled to an RI detector (Spectra Physics, SP 8430). The eluent used was phosphate buffer (0.1M NaH₂PO₄·2H₂O, set to pH 7 by means of 1 M NaOH). The absorption at 520 nm was measured by means of a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer. Centrifuging was carried out in a Sorvall RC-5B apparatus.

The reaction mixtures of the polysaccharides were worked up by pouring them into 96% alcohol (70% of the volume of reaction mixture), which caused the product to precipitate. The white precipitate was centrifuged, taken up in ethanol/water (70/30 v/v), centrifuged once more, taken up in 96% ethanol and centrifuged again. The product obtained was dried under reduced pressure.

The content of glucuronic acid was determined with the aid of the colorimetric uronic acid assay of Blumenkrantz and Abdoe-Hansen, *Anal. Biochem.* 54, 484 (1973) using *m*-hydroxybiphenyl. A calibration curve was produced for polygalacturonic acid (5, 10, 15, 20 μ g). In each case, samples of 20 μ g of the reaction products were measured, of which the percentage of glucuronic acid was determined on the basis of the calibration curve. The carboxyl content of the product was determined by titration of 0.2 g of product with a 0.10 M NaOH solution, alternatively by adding an excess of 0.1 M calcium acetate solution and back-titration of the acetic acid liberated, with 0.10 M NaOH. The relative molecular weight was determined by means of HPLC. The products were further characterised with the aid of NMR.

Example I

Water-soluble potato starch (dry weight 2 g, 12.3 mmol anhydro-glucose unit (AGU)) was dissolved in water (200 ml). Then TEMPO (1% by weight, based on the polysaccharide (0.02 g, 0.13 mmol)) was added and dissolved in approximately 20 minutes. Then 1.5 g (14.6 mmol) of sodium bromide were added and the solution was brought to 0°C. A solution of hypochlorite (45 ml, 4% strength solution, 25.2 mmol) was brought to pH 10.8, using 3M HCl, and cooled to 0°C. The solution was added all at once to the solution of polysaccharide and TEMPO. The progress of the reaction was followed with the help of the alkali consumption, which is equivalent to the uronic acid formation (see Figure 1). During the reaction, the temperature rose to at most 5°C. After completion of the reaction, the mixture was worked up and analysed as stated above. The results are shown in Table 1 below. The percentage uronic acid is a measure of the selectivity of the reaction: since starch has a proportion of 96% of glucose units having a free CH₂OH group, the theoretical maximum for the uronic acid content is 96%. Of the product oxidised at pH 10.8, a ¹³C NMR spectrum (101 MHz) was recorded; the absorption at 177 ppm is characteristic for the primary alcohol function at C-6 (see Figure 3).

Table 1. Yield and uronic acid content of worked-up products.

pH	Reaction time (min)	Yield (%)	Consumption ml 0.5M NaOH ^a	Uronic acid (%) ^b	COOH (%) ^c
9.3	240	99	24.3	86	-
9.8	50	90	25.6	87	-
10.3	35	97	23.3	94	-
10.8	30	96	24.1	92	92
11.3	40	89	23.5	95	-

a: Total number of ml 0.5M NaOH added, before the oxidation was stopped.

b: Determined according to uronic acid assay. The reference was polygalacturonic acid.

c: Calculated by titration with Ca acetate using the molecular weight of polyglucuronic acid (therefore M monomer = 176).

Example II

Example I was repeated, except that the pH was varied. The effect of the pH on the course of the reaction is shown in Figure 2. The results are shown in Table 1. HPLC analysis showed that, at a pH above 11.5, considerable breakdown of the polymer took place.

Example III

Example I was repeated, except that instead of 0.02 g of TEMPO, 0, 0.002, 0.005 and 0.01 g, respectively, of TEMPO were used. The course of the reaction is shown in Figure 4.

Example IV

Example I was repeated, except that instead of 1.5 g of sodium bromide, 0, 0.02 and 0.5 g, respectively, of sodium bromide was used. The course of the reaction is shown in Figure 5.

Example V

Amylose (dry weight 3 g, 18.5 mmol) was suspended in 200 ml of water. The suspension was admixed with 0.03 g of TEMPO (0.19 mmol) and 1.5 g of sodium bromide (14.6 mmol). The suspension was brought to 0°C, and 65 ml of 4% strength hypochlorite having a pH of 10.6, at 0°C, were added. By means of a pH-stat, the pH was maintained at 10.6 during the reaction by the addition of 0.5M NaOH. The reaction was terminated after 3 hours at 0°C by the addition of an excess of ethanol. At that time, 30.5 ml of 0.5M NaOH had been added. The mixture was worked up in the manner described hereinabove. Yield: 92%. Uronic acid content: 75%.

Example VI

Wheat starch (dry weight 3 g. 18.5 mmol) was suspended in 200 ml of water and oxidised in the manner according to Example V. The reaction was terminated after 2 hours at 0°C by the addition of ethanol. At that time, 32 ml of 0.5M NaOH had been added. After working up in the manner described hereinabove, a yield of 94% and a uronic acid content of 81% were found.

Example VII

Various carbohydrates (see table 2) were oxidised using the following conditions: the carbohydrate (20 mmol) (primary alcohol), TEMPO (0.13 mmol, 0.02 g) and NaBr (7.8 mmol, 0.8 g) were dissolved in water (500 ml). A 15% sodium hypochlorite solution (2.2 mol NaOCl per mol of primary alcohol, 10% excess) was adjusted to the desired pH by adding 4M aqueous HCl. Both solutions were brought at the desired temperature. The hypochlorite solution was added at once to the other solution. The pH was controlled with a pH-stat by adding 0.5M NaOH. In this way the formation of acid during the reaction was monitored. The uronate contration was followed by subjecting aliquots taken during the reaction to the colorimetric assay. A linear relation between uronate production and hydroxide consumption was found.

When the oxidation was finished, the reaction was quenched by adding 98% ethanol (10 ml) and the reaction mixture was brought to pH 7 by adding 4M HCl. The polysaccharides were isolated by adding ethanol until a white precipitate formed. The precipitate was centrifuged and washed several times with ethanol/water (70/30 v/v). The product was dried under reduced pressure at 50°C. The oxidised glucose derivatives were dried under reduced pressure and analysed without further purification. The identity of the products was confirmed by ¹H and ¹³C NMR analysis. No other products than the uronic acids could be detected. The results are shown in Table 2.

Table 2

carbohydrate	pH	temp. °C	time min. ^a	yield % ^b	sel. % ^c
methyl- α -D-glucopyranoside	10.0	2	55	n.i. ^d	>95
methyl- β -D-glucopyranoside	10.0	2	35	n.i.	> 95
α , α -trehalose	10.0	2	50	n.i.	> 95
potato starch	10.8	2	80	98	> 95
linear dextrin	10.0	20	45	88 ^e	> 90
pullulan ^f	10.5	2	70	95	> 90
inulin	10.8	2	60	85	> 90

a: time necessary for consumption of 1 mmol OH⁻ per mmol of primary alcohol.

b: yield calculated with molecular weight of oxidised product as sodium salt of polyuronic acid.

c: percentage of oxidation of primary alcohols with respect to total oxidation.

d: not isolated.

e: some loss due to only partial precipitation with ethanol.

f: pullulan mainly consists of repeating (6- α -D-Glcp1-4- α -D-Glcp1-4- α -D-Glcp1-) units; in agreement with this structure, an NaOH consumption of 0.70 mmol per anhydroglucose unit was found.

Example VIII

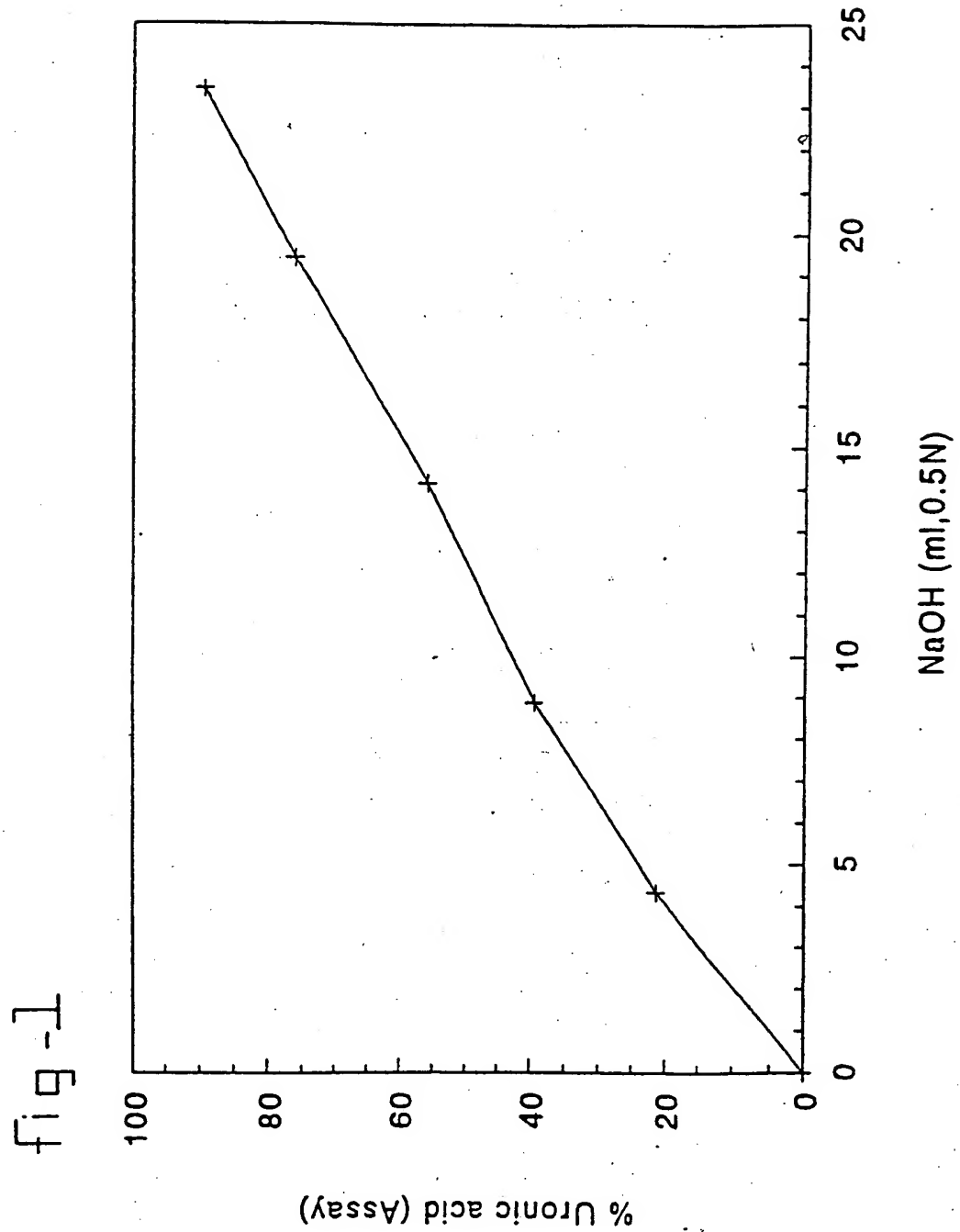
The oxidation of α -methylglucoside according to example VII was repeated, but with varying pH. Table 3 shows the relative reaction rates as a function of the pH, with the reaction rate at pH 10 as 1.0.

Table 3

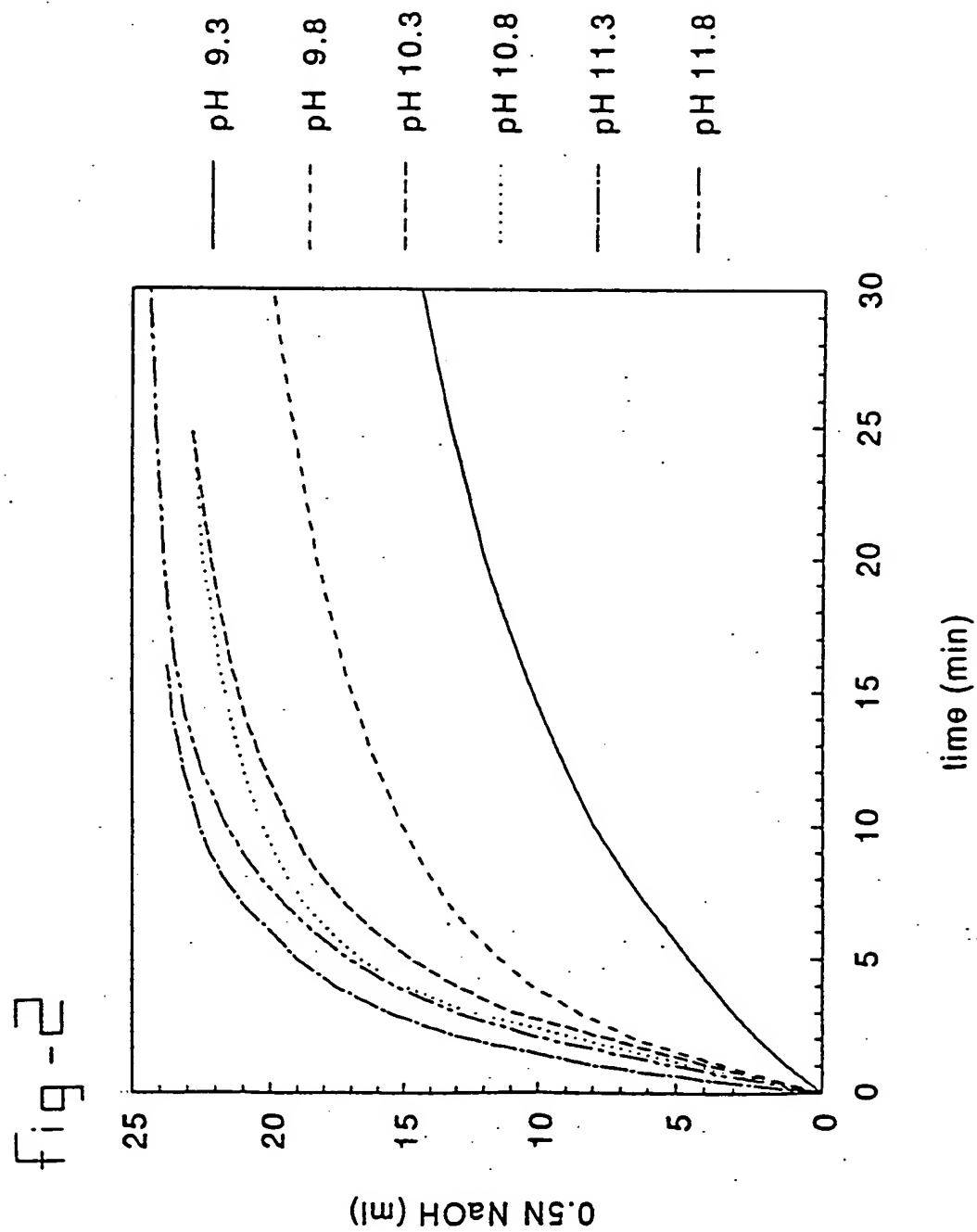
pH	8.0	8.5	9.0	9.5	10.0	10.5	11.0
rel. reaction rate	0.11	0.14	0.17	0.41	1.0	1.0	1.0

CLAIMS

1. Method for oxidising carbohydrates having a primary hydroxyl group, by means of a hypohalite in the presence of a catalytic amount of a di-tertiary-alkyl nitroxyl, characterised in that the carbohydrate is oxidised in an aqueous reaction medium at a pH of between 9 and 13.
2. Method according to Claim 1, wherein the catalytic amount of nitroxyl is 0.1 - 2.5% by weight, based on the carbohydrate.
3. Method according to Claim 2, wherein the di-tertiary-alkyl nitroxyl is 2,2,6,6-tetramethylpiperidin-1-oxyl.
4. Method according to any one of claims 1-3, wherein a pH of 9.3-12 is employed.
5. Method according to Claim 4, wherein a pH of 9.8-11.5 is employed.
6. Method according to any one of Claims 1-5, wherein the carbohydrate is oxidised in the presence of hypobromite.
7. Method according to any one of Claims 1-6, wherein the carbohydrate is oxidised at a temperature of between -5°C and +10°C.
8. Method according to any one of Claims 1-8, wherein the carbohydrate is starch or inulin, or a fraction, a hydrolysis product or a derivative thereof.
9. Poly- α -glucuronic acid obtainable by means of the method according to any one of Claims 1-8, which has a chain length of at least 15 anhydroglucose units and in which at least 90% of the primary alcohol groups have been converted to carboxyl groups.
10. Poly-fructuronic acid obtainable by means of the method according to any one of Claims 1-8, which has a chain length of at least 15 anhydrofructose units and in which at least 75% of the primary alcohol groups have been converted to carboxyl groups.



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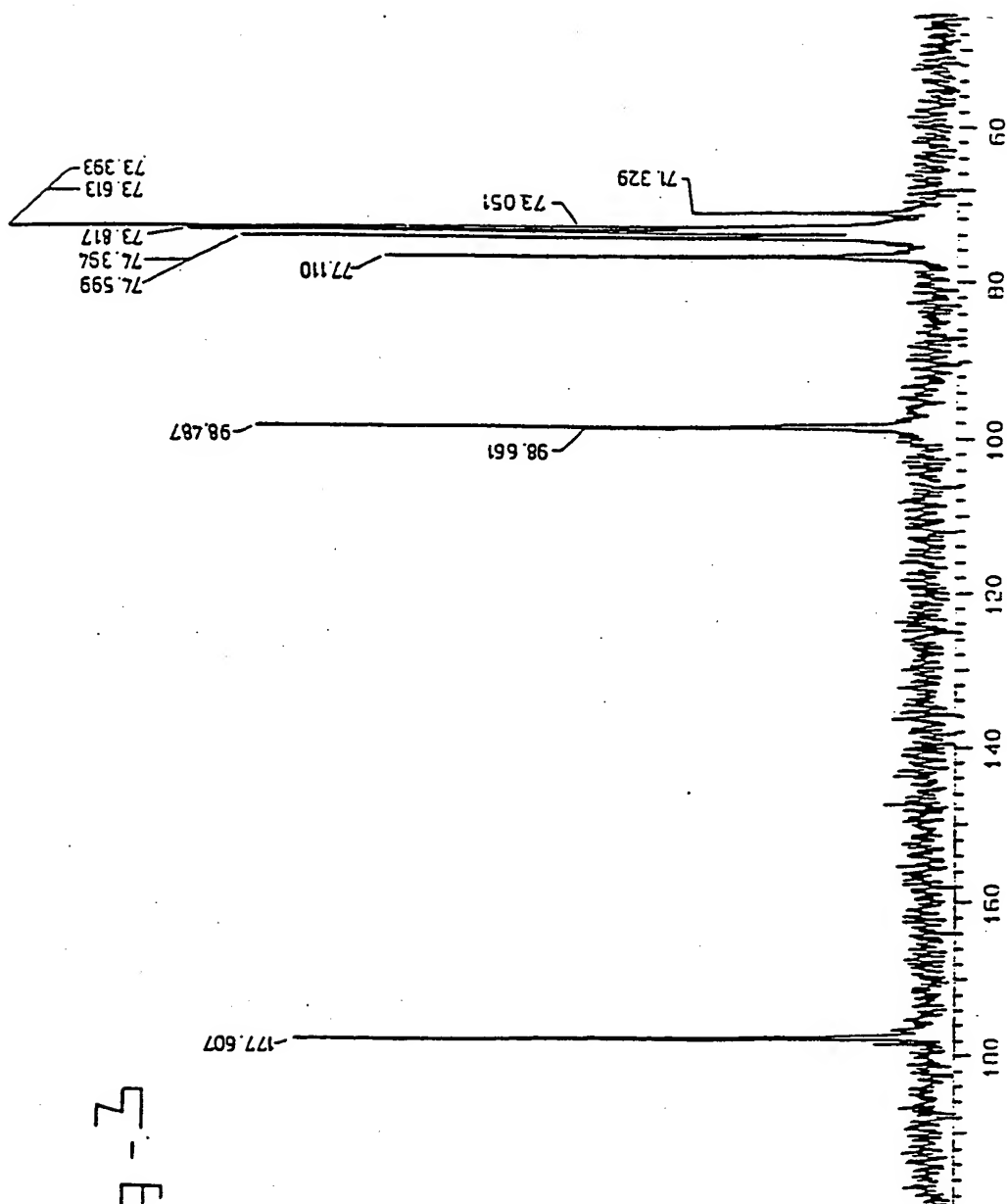
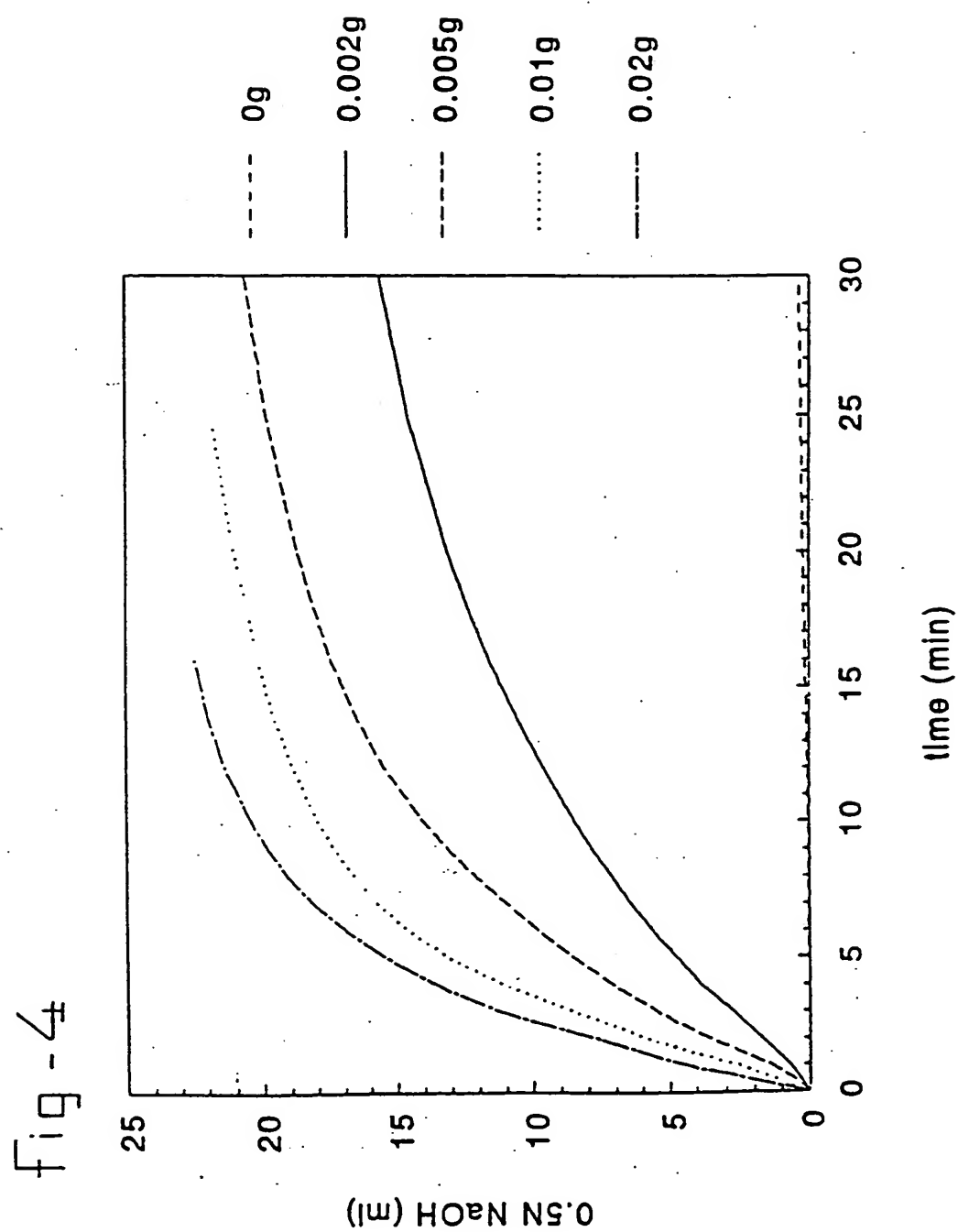


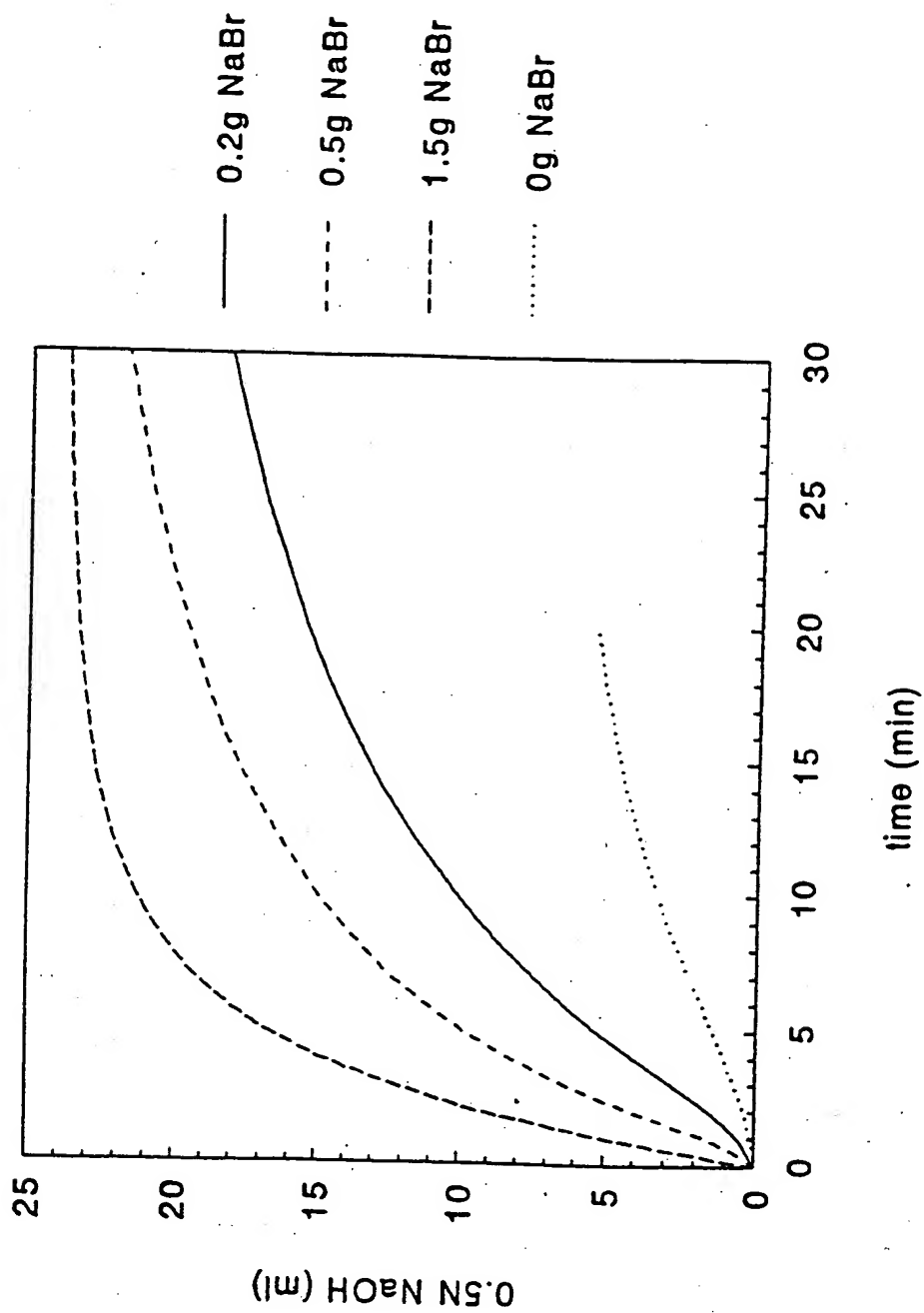
fig - 3

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fig-5



INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 94/00217

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B37/00 C08B31/18 C08B37/18 C07H1/00 C07H7/033

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C08B C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS, vol.113, no.3, March 1994, DEN HAAG NL pages 165 - 166 A.E.J. DE NOOY ET AL. 'Highly selective tempo mediated oxidation of primary alcohol groups in polysaccharides' see the whole document ---	1-10
Y	TETRAHEDRON LETTERS, vol.34, no.7, 12 February 1993, OXFORD GB pages 1181 - 1184 N. J. DAVIS & S. L. FLITSCH 'Selective oxidation of monosaccharide derivatives to uronic acids' cited in the application see the whole document ---	1-10
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

28 December 1994

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FR,A,2 674 528 (SANDOZ S.A.) 2 October 1992 see claims 1-6,15,16 see page 11, line 5 - page 12, line 16 see page 13, line 3 - line 7 see page 13, line 22 - line 27 see page 14, line 15 - line 34 see page 18, line 2 - line 14 see example 8	1-10
Y	JOURNAL OF ORGANIC CHEMISTRY, vol.54, 1989, EASTON US pages 2970 - 2972 P. L. ANELLI ET AL. 'Oxidation of diols with alkali hypochlorites catalyzed by oxammonium salts under two phase conditions' cited in the application see page 2971, left column, line 4 - line 23	1-10
A	CHEMICAL ABSTRACTS, vol. 96, no. 24, 14 June 1982, Columbus, Ohio, US; abstract no. 201463j, see abstract & KHIM. DREV., no.2, 1982 page 114 I. E. ARAKIN ET AL. 'Acceleration and increasing the selectivity of single-stage oxygen soda pulping by addition of an iminoxyl radical'	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 94/00217

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2674528	02-10-92	US-A- 5334756	02-08-94
		CH-A- 683525	31-03-94
		DE-A- 4209869	01-10-92
		GB-A- 2257149	06-01-93
		JP-A- 5194334	03-08-93
		NL-A- 9200556	16-10-92
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